

Article

Novel Aerosol Phase Extraction Method for the Determination of Ca, K, Mg and Na in Biodiesel Through Inductively Coupled Plasma Atomic Emission Spectrometry

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Abstract

A novel extraction method was developed, optimized and validated for the elemental analysis of organic samples. The method, called aerosol phase extraction (APE), was based on the nebulization of the extracting aqueous solution (0.1 mol L^{-1} nitric acid) on the sample. The extraction was performed at the interface of each generated extractant droplets as they entered in contact with the samples. Afterwards, the phases were allowed to separate and Ca, K, Na and Mg were determined in aqueous phase by means of inductively coupled plasma optical emission spectroscopy (ICP-OES). Measurement of aerosol characteristics demonstrated that a water-in-oil emulsion was generated. Therefore, once the aqueous solution was dispersed into the sample, the phases spontaneously separated. Furthermore, the interfacial specific surface area took values on the order of $1 \text{ m}^2 \text{ mL}^{-1}$, hence enhancing the extraction kinetics over a conventional extraction method. The key variables affecting the extraction yield were: the nebulization gas flow rate, liquid flow rate, extraction time, acid concentration, nebulizer tip to sample surface gap and $m_{\text{Organic phase}}/m_{\text{Aqueous phase}}$ ratio. Once the optimum conditions were selected, the method was applied and validated for the determination of Ca, K, Na and Mg by ICP-OES in 0.5 mL biodiesel samples with an expanded uncertainty lower than 2%. With the APE method, the extraction time was around 1 minute, whereas conventional methods employed to perform this kind of extraction required from 4 to 50 minutes. Additionally, the APE involved the analytes preconcentration thus lowering the limits of detection down to the ng mL^{-1} level (*i.e.*, LODs based on the $3 s_b$ criterion were 32, 20, 19 and 24 ng mL^{-1} for Ca, K, Na and Mg, respectively). Furthermore, accuracy when quantification of Ca, K, Na and Mg concentration using APE was not significantly different as compared to that afforded by

conventional liquid-liquid extraction. Finally, Ca, K, Na and Mg contents were determined in four real samples in the 0.5-13 mg kg⁻¹ range. The obtained results were not statistically different to those encountered with a microwave-based digestion method.

Keywords

ICP-OES; Liquid – liquid Extraction; Aerosol phase extraction; Metals; Biodiesel

Highlights

A novel rapid aerosol phase extraction (APE) procedure has been applied for the first time to determine metal impurities in biodiesel samples.

A water-in-oil emulsion is generated during the extraction process and the phases separation is spontaneously and quickly produced.

The method provides high extraction yields with a concomitant analyte preconcentration.

As the APE avoids introduction of organic solvents into the plasma, calibration can be performed with a set of plain acidified water standards.

Novelty Statement

The novel Aerosol Phase Extraction method has been applied for the first time to the analysis of organic samples. In this case, the analytes (Na, K, Ca and Mg) are extracted in an aqueous 0.1 mol L⁻¹ nitric acid solution. The determination of these metals is finally carried out in inductively coupled plasma optical emission spectrometry by applying external calibration with aqueous standards.

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64 Introduction

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66 Biodiesel is a well-established alternative to fossil fuels. Biodiesel quality control
67 involves, among others, the determination of alkaline earth elements (Na, K, Ca, Mg)
68 content.¹ These species are a result of the biodiesel production process and play a very
69 important role because they may modify the efficiency of the biodiesel production as
70 well as its stability. Moreover, the presence of these elements may cause engine damage
71 by corrosion and catalyst poisoning. These elements are present at variable
72 concentrations depending on factors such as the raw materials, production process and
73 the post-production pollution, among others.

74 The quantification of alkaline and alkaline earth elements in biodiesel has
75 several difficulties associated: (i) some of them are present at very low concentrations
76 ($\mu\text{g L}^{-1}$); (ii) there are limited certified reference materials for method validation and
77 quality controlling; and, (iii) the matrix is complex and its composition depends on the
78 biodiesel origin and treatment.

79 Therefore, it is obvious that sensitive techniques are required to carry out the
80 determination of Na, K, Ca and Mg in this type of samples. The main techniques
81 employed are flame atomic absorption spectrometry (FAAS), graphite furnace atomic
82 absorption spectrometry (ETAAS), inductively coupled plasma optical emission
83 spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS).²
84 Dilution in an organic solvent (*i.e.* ethanol, xylene or kerosene) has been widely
85 recommended for the determination of Na, Ca, K and Mg by atomic absorption
86 spectrometry or ICP-OES.^{3,4,5,6,7,8} Unlike FAAS, the latter technique has multielemental
87 capability, it provides lower limits of detection and wider dynamic ranges than the
88 former. However, the introduction of organic samples into the plasma is a challenging

subject as ICP techniques suffer from severe interferences caused by complex organic matrices (e.g., matrix effects, plasma degradation or soot deposition at the injector tip).^{9,10,11,12} To circumvent them, several sample preparation approaches have been developed such as acid digestion and sample oxidation or combustion.^{2,13,14} However, these methods show some problems caused by the addition of reagents, sample contamination, degradation of limits of detection and volatile compounds losses.

Emulsification has been proposed as an alternative to sample dilution, because the mass of organic solvent that reaches the plasma is minimized.¹⁵ Emulsification involves the addition of an aqueous phase containing an acid and/or surfactant to the sample. The selection of the surfactant and its concentration are the most crucial points. On this subject, it is necessary to consider that the emulsion physical properties may affect the nebulization process,¹⁶ the analyte transport efficiency and the stability of the emulsion. By mixing water, oil and one or more surfactants under controlled experimental conditions, either a cloudy (i.e., emulsion) or a transparent (i.e., microemulsion) mixture is obtained.¹⁷ To prepare an emulsion or microemulsion, the surfactant is first dissolved in water and then it is added to the sample. The dispersion is prepared by vigorously stirring or by using an ultrasonic bath.^{17,18} The major drawback of this methodology is related with the loss of sensitivity and the degradation of LODs as the sample is diluted.

Solvent extraction is one of the most versatile and widely used procedures for the removal, separation and concentration of metals.^{19,20} In the last years, research efforts have been focused on the development of efficient, fast and miniaturized extraction methods.^{20,21} In classical liquid–liquid extraction, small droplets are generated by agitation, which increases the contact surface area and improves the extraction efficiency. However, in some instances, the classical procedure is not

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3 114 effective enough and presents some disadvantages: laborious manipulation, large
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5 115 volumes of solvents required, high operational costs, possible formation of emulsions,
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7 116 large equipment and long analysis time. One of the means for improving the extraction
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9 117 efficiency is to increase the medium temperature and/or to apply sonication. This
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11 118 methodology has been satisfactorily used for the determination of Ca, K, Mg and Na in
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13 119 biodiesel by ion chromatography.²² However, complex procedures, large volumes of
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15 120 solvents and long analysis time are still required. In the dispersive liquid-liquid
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17 121 microextraction (DLLME) method, a dispersing agent is added to the extracting one.
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19 122 The extraction efficiency is improved due to the increase in the exchange surface as a
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21 123 substantial number of small droplets of the extracting solvent are dispersed into the
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23 124 liquid sample.^{23,24}

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27 125 In this paper, a new liquid-liquid extraction method, based on the concept of
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29 126 aerosol extraction, previously developed in our laboratory,²⁵ has been applied for the
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31 127 first time to the determination of Ca, K, Mg and Na in organic samples (*c.a.*, biodiesel).
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33 128 The Aerosol Phase Extraction (APE) method is based on the confinement of an aerosol,
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35 129 previously generated from the extraction solution, into the sample. As indicated in
36
37 130 previous reports,²⁶ the extraction has been carried out by using a diluted nitric acid
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39 131 solution, thus eliminating the aforementioned drawbacks associated to the introduction
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41 132 of organic matrices into ICP-OES.
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47 134 **Materials and methods**

48 49 135 50 51 136 *Chemicals and samples*

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54 137 Ultrapure water was supplied by a three-step ion-exchange system Milli-Q, fed by
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56 138 reverse osmosis, Elix 3, both from Millipore (El Paso, TX, USA). 65% HNO₃
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(Suprapur®, Merck KGaA, Darmstadt, Germany) was used to prepare washing solutions and acidify the standards and samples. An ICP multielement standard solution (Merck IV, Merck KGaA, Darmstadt, Germany) containing 1000 mg element per litre was used to prepare the standards by serial dilutions. Stock and standard solutions were prepared in 2 % (v/v) HNO₃.

To evaluate the feasibility of the developed method four real samples were analyzed. A biodiesel certified reference material (CRM), VHGB100M5-10-100G, produced by VHGLabs (Manchester, U.S.A.) was also studied. The CRM had a Fatty Acid Methyl Ester matrix (B100) and the certified Na, Ca, Mg, K and P concentrations were $10.0 \pm 0.1 \mu\text{g g}^{-1}$.

Aerosol phase extraction procedure

Figure 1 shows the experimental set-up used to perform the APE procedure. 0.5-0.9 g of biodiesel were poured into a 5 mL extraction vial. Then, a HNO₃ solution was delivered to a glass pneumatic concentric nebulizer (TR-30-A2, Meinhard®, USA) through a peristaltic pump (Perimax, Spetec, Erding, Germany). The generated droplets were thus directed towards the organic phase. The metal extraction occurred at the interface of each generated droplet, thus giving rise to a large contact surface area. Once the solutions were separated, the Ca, K, Mg and Na concentrations were determined in the aqueous phase through ICP-OES.

All the experiments and measurements were carried out at room temperature. Three extractions and ten measurement replicates were performed for all the evaluated conditions.

Volume drop size distributions and ICP-OES measurements

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3 164 The aerosols generated by the nebulizer were measured by means of a laser Fraunhofer
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5 165 diffraction system (model 2600c, Malvern Instruments, Malvern Worcestershire, UK).
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7 166 The sizer was equipped with a 63 mm lens focal length, which enabled the system to
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9 167 measure droplets with diameters included within the 1.2 to 118 μm range and provided
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11 168 the aerosol liquid volume fraction for a set of 31 diameter intervals (bands) thus giving
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13 169 rise to the complete volume drop size distribution. The nebulizer tip was set at 30 mm
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15 170 from the lens and at 15 mm from the laser beam center.
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18 171 An axially viewed Agilent 720 ICP-OES spectrometer (Santa Clara, USA) was
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20 172 used to determine the intensities for the selected elements. The system was equipped
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22 173 with a 40.68 MHz free-running generator and a polychromator with an echelle grating.
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24 174 A glass pneumatic concentric nebulizer (TR-30-K2, Meinhard®, USA) fitted to a
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26 175 cyclonic spray chamber was used as sample introduction system. Table 1 summarizes
27
28 176 the operating conditions.
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33 34 178 **Results and Discussion**

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37 38 180 *Fundamental studies*

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40 181 In the Aerosol Phase Extraction (APE) procedure, the extracting (aqueous) aerosol was
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42 182 pneumatically generated through the interaction between the liquid stream and a high
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44 183 velocity gas stream. For a given pneumatic nebulizer, the experimental variables that
45
46 184 influence the aerosol drop size distribution are the gas and liquid flow rates. The
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48 185 efficiency of liquid-liquid extraction, in turn, is precluded by the total liquid interface
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50 186 area. The higher this magnitude, the higher the extraction yield. To accomplish this in
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52 187 the case of the APE method, aerosols as fine as possible must be generated. It was
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54 188 clearly observed that at low gas flow rates (*i.e.*, 0.2 L min^{-1}) the proportion of liquid
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3 189 volume contained in coarse droplets was significantly higher than at higher values of
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5 190 this variable. This was a direct consequence of the increase in the gas kinetic energy.²⁷
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7 191 As regards the liquid flow rate, Q_l , an increase in this variable led to a drop in the liquid
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9 192 to gas volume ratio thus leading to coarse aerosols.

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11 In the APE, the metals extraction took place at the interface of each particular
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13 droplet what resulted in a larger contact surface area than in the case of the classical
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15 extraction method. The aerosol surface area distribution was thus obtained by applying
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17 the following equation to each diameter range:
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$$A_i = \frac{600 \times V_{AP} \times \bar{Q}}{\bar{D}} \quad \text{Equation 1}$$

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28 where, A_i is the absolute area for a given band drop diameter (cm^2); V_{AP} is the total
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30 volume of extracting solution required for a complete run, *e.g.*, 0.5 mL; \bar{D} is the
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32 diameter at each band midpoint (μm); and, \bar{Q} is the % aerosol volume at the midpoint
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34 of the band provided by the sizer.
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38 204 Figure 2 shows the absolute surface drop size distributions obtained after
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40 applying Equation 1 at the three gas flow rates studied. It was first observed that a
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42 significant liquid surface was contained in droplets with diameters lower than 1.2 μm (7
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44 and about 20% for 0.2 and 0.4 L min^{-1} , respectively). When considering the curves for
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46 diameters above this value, it was found that they peaked towards lower drop diameters
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48 as increased the gas flow rate (Figure 2).
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52 210 By integrating the curves in Figure 2 it was possible to determine the effective
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54 liquid surface or liquid-liquid interfacial surface area. The obtained values were 0.33,
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56 0.82 and 0.92 m^2 per cm^3 of liquid at Q_g values of 0.2, 0.4 and 0.6 L min^{-1} , respectively
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58 It was therefore verified that the higher the gas flow rate, the higher the interfacial area.
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214 Taking into account a 0.5 mL total volume of extracting solution, the respective total
215 interfacial surface values were 0.16, 0.41 and 0.45 m².

216 The effect of the liquid flow rate was also evaluated. Aerosol measurements
217 demonstrated that at 0.3 and 0.5 mL min⁻¹, about 25% of the liquid surface was
218 contained in droplets with diameters below 1.2 µm; whereas, at 1.0 mL min⁻¹ the
219 surface area contained in droplets with diameters lower than 1.2 µm decreased down to
220 3%. According to these data, the interface surface areas were 0.95, 0.83, 0.41 and 0.28
221 m² per liquid cm³ at 0.25, 0.50, 0.75 and 1.0 mL min⁻¹, respectively.

222 Finally, it was verified that the aerosols generated were composed by droplets
223 whose diameters ranged from 1.2 to around 100 µm. This size range corresponded to a
224 conventional emulsion.²⁸ In conclusion, the presented method was based on the
225 generation of an emulsion without the need of using a dispersing agent. This fact was
226 advantageous, because, due to the instability of emulsions, in comparison with
227 microemulsions,^{29,30} both phases could separate spontaneously.

229 *Optimization of the operating variables*

230 A univariate optimization of the variables affecting the extraction efficiency was carried
231 out. The parameters considered were those that affected the characteristics of the
232 generated aerosols at the sample surface (the nebulizer gas and liquid flow rates and
233 nebulizer tip – sample gap) as well as those that influenced the status of the extraction
234 equilibrium (nitric acid concentration and mass phases ratio). In order to perform this
235 study, Na, K, Ca and Mg concentrations were determined in the biodiesel CRM. For a
236 given element, the recovery (R, %) was given by:

$$238 \quad R(\%) = \frac{C_{det} m_{ext}}{C_{cert} m_{CRM}} \times 100 \quad \text{Equation 2}$$

239

240 where C_{det} corresponded to the experimentally determined analyte concentration, m_{ext}
241 was the mass of extracting solution and C_{cert} and m_{CRM} were the CRM concentration and
242 mass (0.5 g), respectively.

243

244 *Effect of the nebulizer gas flow rate, Q_g*

245 Regarding the effect of the gas flow rate (Q_g), it was possible to anticipate that the finer
246 the generated aerosols (*i.e.*, the higher the interfacial surface), the better recoveries.
247 Thus, according to the data shown in Figure 2 the best conditions would correspond to
248 the highest Q_g evaluated (*i.e.*, 0.9 L min⁻¹). Figure S1 plots the recovery versus the
249 nebulizer gas flow rate showing an unexpected optimum (100%) at 0.4 L min⁻¹. Higher
250 values of this variable induced a drastic drop in the extraction yield. For example, at 0.9
251 L min⁻¹ only 20% of the analyte mass initially present in the sample was extracted. This
252 could be explained by an increase in the speed at which the aerosol impacted against the
253 organic phase. Indeed, it was visually observed that above 0.4 L min⁻¹, a fraction of the
254 sample was ejected from the sample vial and it was consequently lost. For this reason,
255 0.4 L min⁻¹ was taken as the optimum Q_g for the remainder of the study.

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257 *Effect of the liquid flow rate, Q_l*

258 Studies on extraction kinetics were carried out by modifying the liquid flow rate (Q_l) set
259 to aspirate 0.5 g of aqueous phase (extracting solution). According to Figure S2, 100%
260 recoveries were obtained at low liquid flow rates (*i.e.*, from 0.1 to 0.5 mL min⁻¹). This
261 parameter dropped down to 65% as Q_l was further increased up to 1 mL min⁻¹. Because
262 the volume of extracting agent was kept constant, this trend was likely due to the
263 inverse relationship between Q_l and extraction time. Additionally, as mentioned before,

aerosols became coarse (with lower liquid surface areas) as the liquid flow rate was increased. It was finally verified that a 60 s extraction time was enough to obtain recoveries close to 100% for the four elements tested. This time was from 3 to 50 times shorter than those required in previous works.^{22,31}

Effect of the distance from nebulizer tip to the sample surface, d

The effect of the nebulizer nozzle sample surface gap (Figure 1) on R was evaluated. It was observed that recoveries were 100% when the distance was set at values of 1.0 or 1.5 cm. Higher d values made a fraction of the aerosol to impact against the vertical walls of the biodiesel container and did not efficiently interact with the sample. Under these circumstances, recoveries ranged from 80 to 90%. When the nebulizer was set at closer distances from the sample, the aerosol droplets impacted against the surface of the organic phase at a high speed. This, together with the effect of the high velocity stream, caused the ejection of a significant sample mass. The direct consequence of this phenomenon was that a portion of the sample was lost and did not interact efficiently with the extracting agent. For instance, for a 0.8 cm nebulizer – sample surface distance, the measured recovery was 90%.

Effect of HNO₃ concentration

Figure S3 shows that the extraction efficiency increased with the acid concentration. The major effect was observed for calcium. For this element, the recovery went from 60% to 100% as the acid concentration was increased from 0.05 to 0.1 mol L⁻¹. In contrast, magnesium exhibited an around 80% extraction yield for the lowest acid concentration tested. For all the elements, a plateau was reached at a 0.1 mol L⁻¹ HNO₃ concentration.

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290 *Effect of $m_{\text{Organic phase}}/m_{\text{Aqueous phase}}$ ratio (r)*

291 Values of this parameter from 0.5 to 3 were chosen to evaluate the analyte pre-
292 concentration effect of the APE method. Figure S4 shows that, for the four elements
293 tested, the intensity in the aqueous phase increased almost linearly with the r value. This
294 trend suggested that the analyte extraction efficiency did not vary as a function of the
295 aqueous to organic phase ratios within the r studied range. It was later verified that
296 recoveries were close to 100% for all the evaluated r values. Therefore, a r value of 3
297 was selected because of the expected decrease in the limits of detection.

298 In order to monitor whether the phase separation step was properly performed,
299 once the extraction was completed, the carbon 193.030 nm emission signal was
300 measured in the extracting phase and a clean diluted nitric acid solution. No significant
301 differences were found between both signals, thus confirming the absence of emulsified
302 biodiesel traces in the extracting solution.

303

304 *Optimized parameters*

305 Table 2 summarizes the optimized operating conditions for the new aerosol phase
306 extraction approach. A new test was done to check whether the analyte extraction was
307 complete under the optimized conditions or not. The biodiesel sample initially exposed
308 to the APE procedure under optimum conditions was subjected to a second extraction
309 step. Ca, K, Mg and Na concentrations were again measured in the new aqueous phase
310 and they were not significantly different from those measured for a blank. As a
311 conclusion, a single procedure was sufficient for the complete extraction of the analytes.

312

313 *Phase separation step*

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3 314 According to previous studies carried out in our laboratories,²⁵ an aqueous sample could
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5 315 be nebulized over an organic solution in order to perform the extraction of
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7 316 molybdenum. It was verified that, once the aerosol interacted with the organic phase,
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9 317 the phase separation took place in ten minutes. This could be associated to the fact that
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11 318 too fine aerosols were generated. In fact, the nebulizer employed in ref. 25 yielded
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13 319 aerosols whose maximum diameter was 15 μm . Presumably, the existence of a
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15 320 significant proportion of fine droplets caused a slow phase separation. Based on these
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17 321 results, a nebulizer able to generate aerosols more compatible with the production of an
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19 322 emulsion was chosen. As mentioned before, under optimized conditions, the maximum
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21 323 aerosol drop diameter was close to 100 μm . As a consequence, the phase separation
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23 324 took place instantaneously once the aerosol generation ceased. This involved a
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25 325 significant shortening in the analysis time and, hence, a ten times increasing in the
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27 326 sample throughput from 5 to 50 h^{-1} .
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328 *Aerosol phase extraction versus conventional extraction*

329 An extraction procedure adapted from a method recently validated for Na, K, Mg and
330 Ca determination in biodiesel samples was taken for comparison purposes.²² To lower
331 the sample and reagent consumption, the extraction procedure was miniaturized. 250
332 mg of each sample were directly weighed into a centrifuge tube with a precision of 0.1
333 mg. Then, 500 ± 0.1 mg of ultrapure water were mixed with 1.25 μL of a 1 mol L^{-1}
334 HNO_3 solution. The mixture was shaken for 1 min in a vortex apparatus (Selecta,
335 Barcelona, Spain). Afterwards, the tube was partially immersed in a thermostatic bath
336 set at 85 $^{\circ}\text{C}$ for 30 min. This tube was then placed for 15 min in an ultrasonic bath
337 (Selecta, Barcelona, Spain) operating at a 50 kHz frequency. After that, it was

centrifuged at 1000 rpm for 5 min. The aqueous phase was separated and diluted with 0.625 \pm 0.0001 g of ultrapure water.

Table S1 gathers the Na, K, Ca and Mg concentrations found for the two extraction procedures in the biodiesel CRM. Both methods provided similar results. However, the aerosol extraction procedure proved to be slightly more reproducible than the reference method. Certainly, the main advantage of the APE over the reference procedure was the significantly shortening in analysis time from 51 min in the case of the former procedure to 1 min for the aerosol phase extraction methodology.

Method validation

A complete in-house validation and uncertainty estimation according to IUPAC³² and EURACHEM³³ guidelines was carried out.

Linearity and working range

Calibration was performed using five mass fraction levels covering from 0 to 6 mg kg⁻¹. To counter potential memory effects, five replicates for each calibration solution were measured in a random order. Coefficients of determination (R^2) in the 0.9987-0.9996 range were obtained for five different calibration curves, and no outlying measurements, i.e. > 3 times the standard error of the calibration function, were found.

Limit of detection and limit of quantification

Ten independent replicate analyses of the Element Blank Oil 75 Viscosity standard (Conostan®, Ponca City, USA) were carried out under repeatability conditions. This standard was selected because it could be considered as a matrix similar to that of biodiesel. The LOD and LOQ were estimated as 3 or 10 times the total standard

deviation (s_T), respectively. Equation 3 was applied to calculate s_T . Total standard deviation includes the standard deviation of all the measurements (s_M) and the standard deviation coming from the CRM ($s_{Sd} = 0.015 \text{ mg kg}^{-1}$). The LOD and LOQ values were around 0.06 and 0.18 mg kg^{-1} (Table 3). The major contribution came from the blank standard.

368

369
$$s_T = \sqrt{s_M^2 + s_{Sd}^2} \quad \text{Equation 3}$$

370

In order to compare the LODs with previous works, they were estimated according to the $3s_b$ criteria, where s_b was the standard deviation of ten background consecutive measurements. In the present study, the blank corresponded to the biodiesel CRM after performing two consecutive APE procedures in order to ensure that the analytes were quantitatively removed. As Table 3 shows, LODs for the APE method in combination with an ICP-OES spectrometer were from 2 to 20 times lower than those found in previous works. Even considering the contribution of the standard deviation coming from the CRM (Equation 3), LODs found in the case of the new APE were lower than those encountered in the already described methods (see Table 3). Obviously, the use of a sensitive technique such as ICP-OES promoted the reduction in LODs over other techniques considered in Table 3 such as ion chromatography or flame photometry.

383

384 *Trueness*

Trueness was evaluated by measuring the Ca, K, Mg and Na concentration for a biodiesel CRM. Three replicate samples were analyzed on two different days. The evaluation of the measurement results on a certified reference material was done by

comparison of the difference between the certified and measured values (Δ_m) with the combined uncertainty of certified and measured value (U_Δ). The absolute difference between the mean measured value and the certified value were calculated according to Equation 4. The expanded uncertainty U_Δ , corresponding to a confidence level of approximately 95 %, was obtained by multiplication of u_Δ by a coverage factor (k), usually equal to 2. The uncertainty u_Δ was calculated, based on Equation 5, from the uncertainty of the certified value and the standard deviation of the measurement result. To evaluate the method performance, Δ_m was compared with U_Δ . As Δ_m was lower than U_Δ for all the elements, it was concluded that there were no statistically significant differences between the values obtained experimentally and the certified concentrations (Table S2).

$$\Delta_m = |c_m - c_{CRM}| \quad \text{Equation 4}$$

$$u_\Delta = \sqrt{s_m^2 + u_{CRM}^2} \quad \text{Equation 5}$$

where c_m is the mean measured value, c_{CRM} is the certified value, s_m is the standard deviation and u_{CRM} is the uncertainty of the certified value.

Repeatability and intermediate precision

Three sub-samples of a CRM sample were measured on 5 different days, using 5 different calibration curves. One-way ANOVA was used to estimate the repeatability and intermediate precision as within-group and between-group standard deviation, respectively. The repeatability of the method was lower than 0.8 % for all the elements, whereas the intermediate precision was lower than 0.11%.

413

414 *Uncertainty estimation*

415 Calibration solutions preparation has a contribution to the final measurement

416 uncertainty through the purity of the standards and the weighing steps. Furthermore, the

417 uncertainty of the purity of the standards has also been assessed to estimate the total

418 uncertainty contribution of the calibration. The relative standard uncertainty of the

419 calibration resulted in 0.6% by taking into account the uncertainty contribution of the

420 preparation of the calibration solution, all additional contributions coming from the

421 stock solution preparation and the uncertainty of the purity of the standards.

422 The relative standard uncertainty contributions related to the repeatability and

423 intermediate precision were obtained applying one-way ANOVA to the 15

424 measurements (equations 6 and 7):

425
$$u_{rep} = \frac{100}{c_{CRM}} \sqrt{\frac{RSD_{rep}^2}{n_{rep}}} \quad \text{Equation 6}$$

426
$$u_{ip} = \frac{100}{c_{CRM}} \sqrt{\frac{RSD_{ip}^2}{n_{days}}} \quad \text{Equation 7}$$

427

428 where c_{CRM} is the mean value of concentration measured for each element, RSD_{rep} is the

429 repeatability, n_{rep} is the number of replicates, RSD_{ip} is the intermediate precision, and

430 n_{days} is the number of days. Moreover, the relative standard uncertainty related to the

431 trueness contribution was estimated by applying Equation 8.

432

433
$$u_t = \frac{100 \times u_{\Delta}}{c_{CRM}} \quad \text{Equation 8}$$

434

The contributions of the calibration, repeatability, intermediate precision and trueness were taken into account for the calculation of the expanded uncertainty (U) of the measurements.³⁴

438

$$U = k \cdot \sqrt{u_{cal}^2 + u_{rep}^2 + u_{ip}^2 + u_t^2} \quad \text{Equation 9}$$

440

where U is the expanded relative uncertainty, k is the coverage factor ($k=2$), u_{cal} is the relative standard uncertainty of calibration, u_{rep} is the relative standard uncertainty of repeatability, u_{ip} is the relative standard uncertainty of intermediate precision, and, u_t is the relative standard uncertainty of trueness. The coverage factor applied was 2 corresponding to the 95 % confidence level.³⁴ Table S3 shows the final expanded relative uncertainty for each element and the contribution of the different uncertainty sources. For all the elements, the expanded relative uncertainty was around 1.5-1.9%. The major sources of uncertainty contribution were related to the calibration and trueness assessment and they represented around 47-56% and 36-46% of the uncertainty, respectively.

451

452 *Analysis of real samples*

With the aim of validating the aerosol phase extraction methodology, the biodiesel certified reference material and four real samples including three biodiesels and a waste cooking oil were analyzed. Concentration values were also calculated by sample acid digestion and subsequent determination by ICP-OES. The samples were treated using the microwave digestion system Start D (Milestone, Sorisole, Italy). Approximately 250 mg of the sample, weighed with a precision of ± 0.1 mg, were transferred to a microwave digestion vessel and then 7 mL of 65% HNO_3 and 1 mL of 30% H_2O_2 were

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460 added. The sample was digested at 200 °C for 30 min. The digests were transferred to
461 graduated glass flasks and diluted to 10 mL with Milli-Q water.

462 To evaluate whether the difference between the concentrations provided by the
463 two tested methods was significantly different, the statistical t-test was applied. As
464 Table 4 shows, no significant statistical differences were observed between the values
465 obtained by the APE and by the digestion procedure.

466

467 **Conclusions**

468 The new aerosol phase extraction (APE) method could be considered a valuable sample
469 preparation method. From the point of view of green chemistry, the use of organic
470 solvents has been avoided and waste generation has been minimized. Moreover, the
471 developed method reduced significantly the time required for a quantitative extraction.
472 Fundamental studies showed that the extraction procedure is based on an emulsion
473 formation, thus phase separation is spontaneously produced. These advantages have
474 been exploited in the determination of Ca, K, Mg and Na in biodiesel samples. The
475 combination of the APE with ICP-OES provides a simple, reliable, accurate and
476 reproducible method whose results are deemed to be of low uncertainty. In fact, the
477 expanded uncertainty values are in the order of 2%. In face of the results, the APE
478 method together with ICP-OES determination could be proposed as an alternative
479 method to the conventional and regulated ones, where the dilution with an organic
480 solvent is recommended.^{3,4,5}

481 Compared with the previously studied method,²⁵ the new APE procedure shows
482 several differences: (i) the phase separation took place quickly and spontaneously. As a
483 consequence, the analysis time was shortened. This was due to the use of a nebulizer
484 able to generate droplets whose diameters matched perfectly within the values
485 theoretically required to generate emulsions (i.e., < 100 μm). In fact, the whole sample
486 preparation took place in around 65 and 630 s for the APE and the previously developed
487 method,²⁵ respectively; (ii) the analyte was extracted in aqueous phase, thus avoiding
488 the problems associated to the introduction of organic solutions into the ICP; (iii)
489 sample losses caused by re-nebulization were minimized as on the one hand the
490 extracting solution was nebulized over the sample and, on the other hand, the nebulizer

tip to sample surface was optimized; and, (iv) it was not necessary to add complexing agents in order to promote the analyte extraction.

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Table 1. Operating conditions of the ICP-OES detector.

Variable	Value
Liquid flow rate, $Q_l/\text{mL min}^{-1}$	0.8
Nebulizer gas flow rate, $Q_g/\text{L min}^{-1}$	0.7
Outer plasma gas flow rate, L min^{-1}	15
Intermediate plasma gas flow rate, L min^{-1}	1.5
Rf power, kW	1.4
Integration time, ms	25
Sampling time, s	1
Elements and Wavelengths, nm	Ca 396.847
	K 766.491
	Mg 279.553
	Na 589.592

Table 2. Optimized operating conditions of the aerosol phase extraction procedure.

Variable	Value
Nebulizer gas flow rate, Q_g , L min ⁻¹	0.4
Nebulizer liquid flow rate, Q_l , mL min ⁻¹	0.3
Extraction time, s	60
HNO ₃ , mol L ⁻¹	0.1
Nebulizer tip to sample surface gap (d), cm	1.5
$m_{\text{Organic phase}}$, g	0.9
$r = m_{\text{Organic phase}}/m_{\text{Aqueous phase}}$	3

Table 3. Limit of detection (mg kg^{-1}) obtained for Ca, K, Mg and Na by applying different extraction and determination procedures.

	APE*	APE [#]	APE ^{&}	Liquid extraction + Flame photometry ^{#,31}	Liquid extraction + Line source flame atomic absorption spectrometry ^{#,31}	Conventional liquid- liquid extraction + Ion chromatography ^{#,22}
<i>Ca</i>	0.06	0.03	0.017	--	0.15	0.23
<i>K</i>	0.05	0.02	0.008	0.18	0.21	0.42
<i>Mg</i>	0.05	0.02	0.015	--	0.03	0.36
<i>Na</i>	0.05	0.02	0.014	0.36	0.04	0.11

* Calculated considering the standard deviation obtained by applying Equation 3.

[#] Calculated considering only the standard deviation of all blank signal measurements.

[&] Calculated considering the standard deviation of the biodiesel sample initially exposed to the APE procedure subjected to a second extraction.

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Table 4. Ca, K, Mg and Na concentration (mg kg⁻¹) obtained by APE and acid digestion methods. Calculated t values, s values, the critical t value ($\alpha=0.05$) and equations employed to calculate t and s.

+	Concentration (Mean \pm s) (mg kg ⁻¹)								Statistical analysis							
	Ca		K		Mg		Na		Ca		K		Mg		Na	
	<i>APE</i>	<i>Digestion</i>	<i>APE</i>	<i>Digestion</i>	<i>APE</i>	<i>Digestion</i>	<i>APE</i>	<i>Digestion</i>	<i>t</i> [*]	<i>s</i> [#]	<i>t</i> [*]	<i>s</i> [#]	<i>t</i> [*]	<i>s</i> [#]	<i>t</i> [*]	<i>s</i> [#]
<i>Biodiesel A</i>	4.16 \pm 0.02	4.3 \pm 0.3	2.07 \pm 0.04	2.31 \pm 0.17	5.805 \pm 0.006	5.85 \pm 0.06	2.538 \pm 0.011	2.60 \pm 0.08	0.9	0.2	2.43	0.12	1.34	0.04	1.38	0.06
<i>Biodiesel B</i>	3.65 \pm 0.03	3.71 \pm 0.2	2.089 \pm 0.007	2.11 \pm 0.03	5.76 \pm 0.02	5.67 \pm 0.12	3.005 \pm 0.014	2.94 \pm 0.08	0.49	0.16	1.61	0.02	1.20	0.08	1.29	0.06
<i>Biodiesel C</i>	1.03 \pm 0.02	1.05 \pm 0.10	2.724 \pm 0.004	2.74 \pm 0.09	6.56 \pm 0.03	6.49 \pm 0.05	2.536 \pm 0.009	2.55 \pm 0.02	0.38	0.07	0.38	0.06	2.22	0.04	1.164	0.017
<i>Waste oil</i>	12.83 \pm 0.08	12.9 \pm 0.5	7.787 \pm 0.016	7.71 \pm 0.05	6.64 \pm 0.02	6.68 \pm 0.02	13.207 \pm 0.010	13.43 \pm 0.18	0.1	0.3	2.58	0.04	2.49	0.02	2.20	0.13
$*t = \frac{(\bar{x}_1 - \bar{x}_2)}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$ $*s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}$ The F-test demonstrated that the variances of both methods procedures were statistically comparable. Critical t value ($\alpha=0.05$): 2.78																

* $t = \frac{(\bar{X}_1 - \bar{X}_2)}{s \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$, # $s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}$, The F-test demonstrated that the variances of both methods procedures were statistically comparable. Critical t value ($\alpha=0.05$): 2.78.

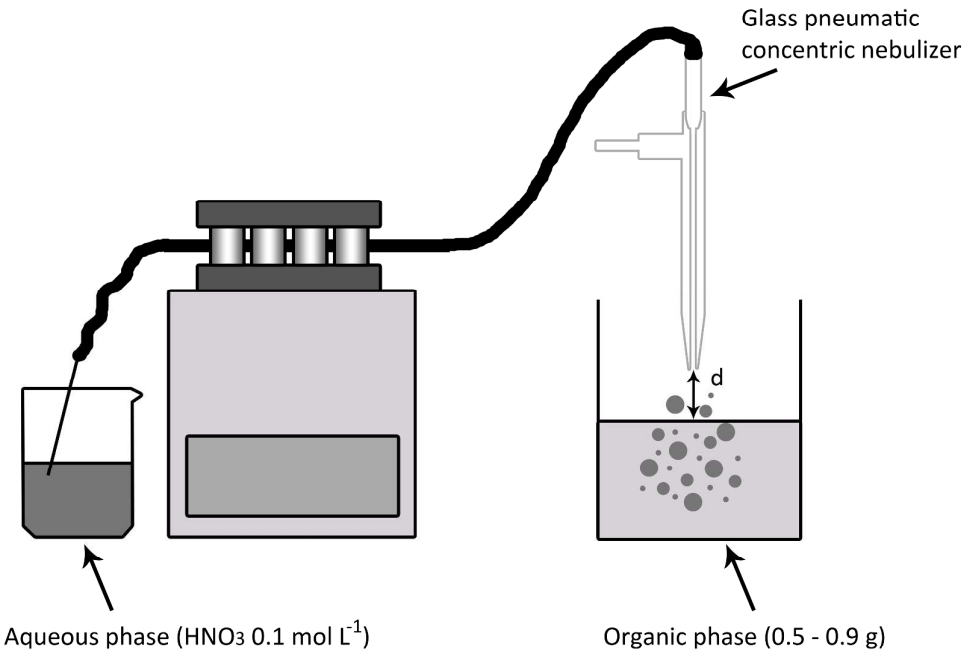


Figure 1. Scheme of the experimental setup employed to perform the Aerosol Phase Extraction, APE.

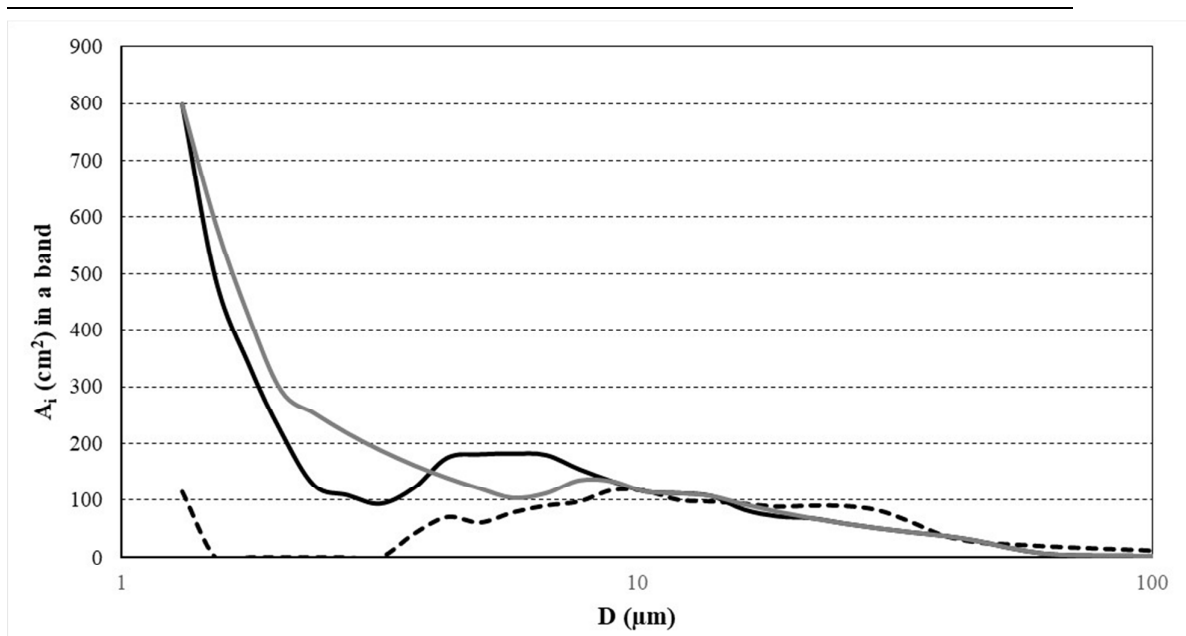


Figure 2. Absolute surface drop size distribution curves in band for the aerosols generated by a concentric nebulizer under different gas flow rates. Liquid flow rate 0.5 mL min^{-1} ; gas flow rate: 0.2 L min^{-1} for dotted line; 0.4 L min^{-1} for black line; and, 0.6 L min^{-1} for grey line.